

The first recorded outbreak of cryptosporidiosis due to *Cryptosporidium cuniculus* (formerly rabbit genotype), following a water quality incident.

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Short title: Rabbit Cryptosporidiosis Outbreak

Abstract

Background

We report the first identified outbreak of cryptosporidiosis with *Cryptosporidium cuniculus* following a water quality incident in Northamptonshire, UK.

Methods

A standardised, enhanced *Cryptosporidium* exposure questionnaire was administered to all cases of cryptosporidiosis after the incident. Stool samples, water testing, microscopy slides and rabbit gut contents positive for *Cryptosporidium* spp. were typed at the *Cryptosporidium* Reference Unit.

Results

Twenty-three individuals were microbiologically linked to the incident although other evidence suggests an excess of 422 cases above baseline. The majority were female, however unusually for cryptosporidiosis there were no affected children under the age of 5 years identified. Mean water consumption was higher than in national drinking water consumption patterns. Diarrhoea duration was negatively correlated to distance from the water treatment works where the contamination occurred. Oocyst counts were highest in water storage facilities.

Conclusions

This outbreak is the first caused by *C. cuniculus* infection to have been noted and has conclusively demonstrated that this species can be a human pathogen. Although symptomatically similar to cryptosporidiosis from *C. parvum* or *C. hominis*, this outbreak has revealed some differences, in particular no children under 5 were identified and females were over represented. These dissimilarities are unexplained although we postulate possible explanations.

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Manuscript

Introduction

Cryptosporidiosis is a diarrhoeal illness caused by species of the protozoan genus *Cryptosporidium*. The faeco-orally transmitted parasite causes infection in many animal hosts and humans. There are at least 23 recognised *Cryptosporidium* species (Fayer and Santín 2009, Fayer *et al.* 2010, Ren *et al.* , Robinson, Guy. *et al.* 2010, Ronald 2010); however two predominate in human disease, *Cryptosporidium hominis* and *Cryptosporidium parvum* (Davies and Chalmers 2009)

Cryptosporidium oocysts, the transmissive stage, shed in faeces, are very hardy and can survive for months in moist environments and resist chlorine disinfection commonly used in water treatment processes. (Davies and Chalmers 2009) They may be found in any faecally contaminated water, including surface, ground and recreational waters. (Davies and Chalmers 2009, Yoder and Beach 2010)

The main symptoms of cryptosporidiosis are watery diarrhoea, abdominal pain, nausea and/or vomiting and low-grade fever. Typically in industrialised countries, symptoms are most common in young children between 1 and 5 years of age. (Chalmers, R.M. *et al.* 2009, O'Donoghue 1995) Although self-limiting, with a typical duration of illness of 2-3 weeks in immune-competent people, *Cryptosporidium* can also cause serious, chronic infection in immunocompromised individuals. Non-*C. parvum* and *C. hominis* species and genotypes are notable in this group (Elwin *et al.* 2011). There is no specific treatment licensed in the European Union. Symptoms (which can be relapsing) persist for up to three weeks (longer in the immunocompromised) (Abubakar *et al.* 2007, Cacciò *et al.* 2005, Davies and Chalmers 2009, Hunter and Nichols 2002).

Outbreaks have been associated with recreational waters, mains and private drinking water supplies, farm visits, childcare and institutional settings and occasionally food. (Alpert *et al.* 1986, Cacciò *et al.*

2005, Davies and Chalmers 2009, Dworkin *et al.* 1996, Hayes *et al.* 1989, Howe *et al.* 2002, Mac Kenzie *et al.* 1994, Said *et al.* 2003)

We report an outbreak of *Cryptosporidium cuniculus* following a water quality incident in Northamptonshire, UK. We focus on the epidemiological characteristics of individuals affected and make comparisons with previous events. The microbiological confirmation of *C. cuniculus* has been described previously (Chalmers, R. M. *et al.* 2009).

The incident

On 25th June 2008, an emergency arose in the potable water supply for central and western Northamptonshire, UK, providing water to a population of approximately 258,000, from a surface water reservoir and treatment works within the county (DWI 2009). The utility provider reported the presence of *Cryptosporidium* oocysts in the potable water supply. Although not statutorily required, the company routinely tested for *Cryptosporidium* from both the raw and treated parts of the system. The process for detecting *Cryptosporidium* is complex (Environment Agency 2010). Detection doesn't indicate the viability of oocysts found, what species they are or their pathogenic potential to humans. For these reasons and also because local immunity contributes to whether there is a hazard posed by the organism, there is no specific UK potable water regulation standard for acceptable counts of oocysts, however, drinking water must not contain parasites at a concentration that could affect human health. Water utilities therefore adopt a risk management based methodology for controlling water supply pathogens informed by the World Health Organisation Water Safety Plan approach to drinking water standards (Davison *et al.* 2005, WUK 2011). In this incident, a small number of oocysts were noted in a single continuous filtering sample taken from the 'final' clean water storage system, between 09.29h on 19th and 11.50h on 23rd June; 6 oocysts were seen in 11848 L (or 0.0005 oocysts / L) water sampled. This was at variance with previous monitoring data (normally none found). Sampling was repeated

between 11.50h on 23rd June and 20.00h on 24th June and revealed a count of 418 oocysts seen in 5064 L (0.08 oocysts / L) water.

Public health authorities and the Drinking Water Inspectorate (DWI – the national water regulator) were notified and control measures rapidly instituted including the dissemination of boil water messages using a variety of routes including local radio and television. (DWI 2009)

Local health professionals were placed on alert for possible cases of cryptosporidiosis occurring in the locality through emergency communications cascade systems and were asked to take stool samples for microbiological analysis and notify suspicious cases to the local health protection team.

Raw water sampling at the time of the initial alert revealed no oocysts and it was therefore considered that the contamination was unlikely to have arisen as a result of overwhelming of the treatment capacity of the plant. As a result the search for the source centred on a possible biosecurity breach from within the treatment works. Oocyst contamination monitoring was expanded to strategic parts of the water distribution network, including end user taps. During the incident the maximum count of oocysts noted was 1.7 oocysts / L (10 L grab sample) on 26th June at a clean water storage reservoir. Sporadic oocysts were found up until 22nd July from storage sites, although counts were below 0.01 / L by 2nd July. Sampling from customer taps, revealed a peak oocyst count of 0.19 oocysts / L (259 oocysts in 1391 L) at one address on 25th June and 0.007 oocysts / L (9 in 1166 L) at another – a 27 fold difference over a similar time frame. Counts at customer taps dropped to below 0.01 oocysts / L by 29th June, but sporadic oocysts were found until 3rd August.

Investigations identified a clean water biosecurity breach. A defect in a vent in a clean water storage tank had allowed a wild rabbit (*Oryctolagus cuniculus*) to enter the clean water side, which had disintegrated and released *Cryptosporidium* oocysts into the water supply. (DWI 2009)

The outbreak

After the incident it was not clear whether there would be any cases of cryptosporidiosis. Existing surveillance systems were used to identify possible cases using the following case definition: Cases of diarrhoea / gastrointestinal illness with microbiologically confirmed *Cryptosporidium spp.* occurring in the water distribution area, having consumed mains water between the dates 19th June until the boil water notice was issued on 06.00hrs on 25th June (later revised to be specific to *C. cuniculus*).

In the 7 weeks following the incident (up to week ending 6th August), 32 microbiologically confirmed cases of cryptosporidiosis were notified to the Health Protection Agency, East Midlands (HPA). Of these, 23 were linked to the incident epidemiologically and microbiologically by typing. As a comparator over the same period, there were only 4 unrelated cases of cryptosporidiosis notified to the HPA from the same geographic area as this incident.

Methods

A standardised, enhanced *Cryptosporidium* exposure questionnaire was administered by telephone, post or in person to all cases of cryptosporidiosis notified to the HPA occurring in the weeks following the incident. Details of symptoms, water consumption, co-morbidities and medication history were obtained. Stool samples positive for *Cryptosporidium* were typed at the *Cryptosporidium* Reference Unit (Chalmers, R. M. *et al.* 2009).

The distance of each case place of residence from the water treatment works was estimated using Microsoft Corp., MapPoint ©, direct 'as the crow' flies and 'by road ' – the latter chosen as water pipes (at least in part) tend to follow road routes.

Statistical analysis

All statistical analyses were undertaken in Stata (StataCorp Inc., version 10) ©. Baseline characteristics of cases were summarized. Interquartile ranges are reported for medians. Statistical significance of difference in means and proportions was calculated with two group mean and proportion tests respectively. Parametric (linear) and non-parametric (Spearman's) regression analyses assessing the association between date of onset of illness and volume of water drunk / distance from the water treatment works were estimated. *P*-values of ≤ 0.05 were considered statistically significant.

Results

23 individuals were linked epidemiologically and microbiologically to the incident (Chalmers, R. M. *et al.* 2009). Little data was available from one (non-responder). The mean age was 32 (26.6 – 37.4) years, (males 33 years, females 32 years, $p = 0.90$). There were 7 males and 16 females (30% male); this difference approached but was not statistically significantly different ($p = 0.061$). At least one of the cases and possibly a second may have resulted from secondary infection.

All cases presented with diarrhoea. The first developed symptoms on 24th June and the last on 14th July. The mean date of onset of diarrhoea was 2nd July (1st July males, 2nd July females, $p = 0.50$).

The monitoring data indicate that the first possible date of exposure was 19th June and the last 23rd / 24th June. However, the count of oocysts per litre increased 165 fold from the sample completed on 23rd June and the one commenced on 23rd June and completed on 24th June and then reduced rapidly. The date of dissemination into the final clean water supply was therefore taken as 23rd June.

Using 23rd June as the exposure date, the incubation period for *C. cuniculus* ranged between 1 and 21 days, mean 9.2 days [95% ci 7.4 -11.0] (males 8.3 [6.3 – 10.2] and females 9.6 [7.1 – 12.1], $p = 0.50$) and median of 8 days [interquartile range [IQR] 8 – 10], (8 males [IQR 7 – 10] and 8.5 [IQR 8 – 9.5] females).

The epidemic curve was similar for men and women (Fig); however there were two late presenting females who did not share address with any of the earlier cases, although one shared an address with a symptomatic individual for whom microbiological confirmation was not obtained.

The median duration of diarrhoea was 13 [IQR 6 – 19] days, (males 5.5 [IQR 5 – 13] days, females, 14 [9 – 20] days).

Other epidemiological characteristics of cases including other potential risk factors are shown in table 1.

The outbreak was classified as being strongly associated with drinking water on the basis that the pathogen identified in clinical cases was also found in water samples from the treatment works (Tillett *et al.* 1998).

Water consumption

The median self-reported total daily mains water consumption was 2.1 L [IQR 1 – 3.3] (male 2.3L [IQR 0 – 3.1], female 1.9L [1.3 – 3.6]), with a median unboiled water consumption of 1.8 L [IQR 0.6 – 2.5] (males 1.8 L [IQR 0 – 2.4], females 1.6 L [IQR 0.75 – 3] and median boiled water consumption of 0.6 L [IQR 0 – 0.9], (males 0.25 L [IQR 0 – 0.9], females 0.6 L [IQR 0.15 – 0.95]).

Mains water consumption (total, cold or boiled) did not correlate to the incubation period or date of onset of diarrhoea. There was also little correlation between duration of diarrhoea and total volume of water drunk (spearman's rho 0.19, p = 0.46), cold or hot (boiled) tap water (spearman's rho 0.24, p 0.29 and - 0.1. p 0.72 respectively).

Distance from water treatment works

The median distance of cases from the water treatment works was 6.24 km [IQR 3.9 – 7.9] 'as the crow flies' and 9.8 km [IQR 5.2 – 12.1] 'by road'. Neither was correlated with the date of onset of illness,

however, duration of diarrhoea was significantly negatively correlated to distance from the water treatment works 'as the crow flies' (Spearman's rho -0.4847, $p = 0.0260$).

Discussion

Most human cases of cryptosporidiosis in the UK are due to *C. parvum* or *C. hominis* with other species appearing occasionally (Chalmers, R.M. *et al.* 2009, Davies and Chalmers 2009, Elwin *et al.* 2011). World-wide, this is the only reported human outbreak of a *Cryptosporidium* species other than *C. parvum* or *C. hominis* (Elwin *et al.* 2011). Prior to this incident, only one case of *C. cuniculus* infecting a human had been reported. (Robinson, G *et al.* 2008) It is now clear that *C. cuniculus* is a human pathogen (Chalmers, R. M. *et al.* 2009). Subsequent investigations have identified sporadic cases in the UK (Chalmers RM *et al.* 2011).

Overall, the incident management decisions taken to control this exposure appear to have been correct. However, there are some observations to note as outlined below.

Incubation period

The calculated incubation period is likely to be subject to error for a number of reasons as outlined below.

The methodology used for obtaining the continuous filtering sample taken between 19th and 23rd June does not allow precise estimation of when the oocysts first contaminated the final water. Contamination could have occurred at any stage in that four day period, although given the 165 fold increase in counts obtained from the following sample taken between 23rd and 24th June, it is reasonable to assume that the major contamination occurred on 23rd June. However, it is possible that some lower level contamination occurred earlier which might explain the otherwise apparently short incubation period experienced by the first case who developed symptoms on 24th June. Additionally, the date of

contamination at the treatment works may not have been the date of exposure as the transit time of water through the distribution system is not uniform nor will water consumption behaviour be the same in all cases. The use of a single exposure date is therefore artificial. Lastly, the peak oocyst counts were noted in the distribution water samples between 25th and 27th June and the mean onset date was 2nd July. It is therefore possible that the incubation period was closer to 7 rather than the calculated 9.2 days. As a result of these uncertainties the estimated incubation period may be under or over estimated. However, in the absence of more precise data on which to base the estimate, other possible incubation period approximations would be speculative.

Children

Sporadic cases of cryptosporidiosis mainly affect children aged one to five years in the UK. Even in waterborne outbreaks, where there is often an increase in adult cases, children are mainly affected. (Davies and Chalmers 2009) For example, in the outbreak of *C. parvum* in Clitheroe, Lancashire, UK, 52% of cases occurred in children < 5 years of age (Howe *et al.* 2002). This outbreak is unusual in that no children in this age group were reported or epidemiologically directly linked. It is not clear why. Biologically plausible explanations could include: the volume of water consumed by young children was insufficient to provide an infectious dose; the alert occurred early in the morning, giving time for parents to protect their children with alternative drinking water sources; adults avoided giving potentially contaminated water to their children, but took less care for themselves.

Nonetheless, recent evidence on unusual cases of cryptosporidiosis indicates that the median age is older in non *C. parvum* / *C. hominis* infection in the UK (Chalmers RM *et al.* 2011, Elwin *et al.* 2011). The data indicate that infection due to *C. cuniculus* matches this pattern. It is thought that with some unusual *Cryptosporidium* species, this may be due to differential exposure e.g. during foreign travel (this explanation does not apply to this outbreak), although the reasons are not fully understood.

Volume of water

The median mains water consumption in cases arising from the incident was 2.1 L [IQR 0 – 3.1]. This is greater than the 2008 Phase-Two (summer) National Tap Water Survey which indicates a mean of 1.329 L of tap water or 2.003 L total fluid consumption per day – including other sources (East 2008). Greater dosing via higher water volumes consumed may provide an explanation for why these individuals were affected.

Distance from the water treatment works

The duration of diarrhoea had a statistically significant negative correlation to the distance ‘as the crow flies’ from the water treatment works. This may have occurred by chance; however, it could be that due to dilution, oocyst counts were lower at points further from the treatment works. (Oocyst viability could have also declined over distance, although we have no evidence to support this.) The monitoring data are difficult to interpret as there was evidence of some concentration of oocysts in clean water storage reservoirs and at customers’ taps but these were variable with some very low oocyst counts taken at similar times. The complex structure of the water distribution system produces variable flows of water over time and therefore possibly unpredictable distribution of pathogens entering the system. Early on in the incident (25 June), there were only 4 end user points tested which may not have been representative of oocyst load at other end users at the time when the loading was likely to be highest. Nonetheless some studies have found that the infective dose magnitude influences the time to and duration of oocyst excretion, but not clinical incubation period or severity of illness (DuPont *et al.* 1995), although others have found longer incubation periods with lower infective doses (Chalmers, Rachel. M. and Davies 2010). It is possible diarrhoea duration is consistent with oocyst excretion duration and therefore the infective dose received might explain this finding.

Sex ratio

The predominance of females in this incident is unusual, although other outbreaks have shown similar sex distributions (Carnicer-Pont *et al.* 2005, Mac Kenzie *et al.* 1994). Although the difference was not significant, it nonetheless approaches significance. If true, there are a number of possible explanations: although men drink more liquid per day than women (East 2008), the latter consume more un-boiled tap water as a proportion of their intake increasing their opportunity for exposure. However, the water consumption of the cases does not support this. Nevertheless it is important to bear in mind the possibility of recall bias in this instance, inaccuracy in personal consumption estimates and assumptions made in the analysis where quantities were not clearly given (e.g. one cup); men are less likely to seek medical advice for health issues so that samples were not taken (Galdas *et al.* 2005, Noone and Stephens 2008); a behavioural explanation, e.g. timings of plain water consumption in males vs. females; an unexplained difference in response to infection between the sexes. The outbreak was caused by *C. cuniculus* GP60 gene subtype Va (Chalmers, R. M. *et al.* 2009). In subsequent investigations it has been found that in sporadic cases the proportion of females affected is greater than males with Va subtype than Vb (Chalmers RM *et al.* 2011).

Infective dose

The volume of water flowing through the distribution system will have diluted the number of *Cryptosporidium* oocysts in any single litre of water (sampling at the water treatment works reached 0.08 oocysts per litre). This supports the generally accepted view that the number of organisms required to be ingested to cause symptomatic infection is very low (Chalmers, Rachel. M. and Davies 2010, DuPont *et al.* 1995). The maximum concentration of oocysts per litre of water in this outbreak at the treatment works was below the former regulation treatment standard of < 1 oocyst per 10L of water

(ceased 22nd December 2007) (DWI 2009), indicating the potential for infection to occur with very low counts.

Surveillance and attack rate

It is perhaps surprising that so few confirmed cases were identified given that approximately 258,000 people were potentially exposed (DWI 2009). Whether the low numbers are testament to the vigorous public health response is unclear. There is however evidence to suggest that the HPA surveillance system only identified a subset of cases. A report for the Consumer Council for Water interviewed individuals affected by the incident and noted that some had been ill, but none had sought medical attention. (2008a) Another study examining syndromic data (NHS Direct data and GP consultations) identified an excess of diarrhoea cases from the area at the time of the incident (Smith *et al.* 2010). Unpublished HPA/Q Surveillance system data (S. Smith, Health Protection Agency, West Midlands, 2011 personal communication) also show an increase in diarrhoea cases for children under 5 years of age in the affected areas, possibly indicating an ascertainment bias in the local reporting of cases. Diagnoses of diarrhoea were 25% higher than normal in the weeks following, with an absolute excess of 422 cases above normal. This is compatible with an established estimate of 15:1 for the true burden of disease for *C. parvum* and *C. hominis* compared with the number of routinely ascertained confirmed cases (Nichols *et al.* 2006, Smith *et al.* 2010). This suggests *C. cuniculus* may have comparable levels of population level impact and should be viewed as a potentially significant cause of waterborne infection.

Hazard

This outbreak has demonstrated the hazard posed by wildlife to the safety of mains water. The DWI although supportive of their handling of the incident, was critical of the water company's maintenance arrangements (2008b, DWI 2009). The DWI also highlighted the importance of oocyst typing to aid in

source identification. Although the cause was found early, had this not been the case, timely knowledge of the *Cryptosporidium* species or genotype would have been helpful for directing control measures.

Boil water notice

A boil water notice was instituted early on 25th June and lifted 4th July. The risk / benefit based decision to remove the boil water notice on 4th July would appear to have been appropriate despite continued sporadic oocyst detections from the distribution network up to and beyond that date as further primary cases occurring after 4th July were not observed, assuming that the two apparent secondary cases were not in fact independently infected from the very low residual counts rather than from an infected contact and that routine surveillance systems did not miss other cases. Nonetheless, continued sporadic oocyst detections in the water distribution network create a dilemma for public health decision makers over what is an appropriate oocyst count to accept for lifting a boil water notice. It cannot be determined from this incident whether similar residual contamination parameters from a future *C. hominis* or *C. parvum* incident could be used to determine when to lift a boil water notice as the differing species will have differing pathogenicity and infectivity patterns in this context.

Human pathogen

Finally, *C. cuniculus* has now been demonstrated to be a human pathogen (Chalmers, R. M. *et al.* 2009). The constellation of symptoms is similar but with some apparent differences to other species, in particular the age and sex profile (table 2) (Davies and Chalmers 2009). However, this pattern has also been found to be the case in recent work investigating the epidemiology of sporadic *C. cuniculus* cases (Chalmers RM *et al.* 2011). It is not possible to conclude from the data whether this is unique to this species or artifactual, but other “unusual” *Cryptosporidium* species differ in their epidemiology from *C. parvum* and *C. hominis* too, although the numbers of cases are small and therefore conclusive differences are difficult to currently ascertain (Elwin *et al.* 2011).

Conclusions

This human outbreak of *C. cuniculus* was the first to have been reported internationally and has demonstrated that the organism is a human pathogen. Some unusual epidemiological features were noted relative to other cryptosporidium outbreaks, in particular the predominance of female cases and the absence of affected children, however it is likely that there was substantial underreporting. The infective dose is likely to be very low (< 1 oocyst per 10L of water), consistent with infection from other *Cryptosporidium* species. Maintenance of potable water biosecurity is important for sustaining the public's health.

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Ethics statement

This manuscript reports the epidemiological findings of the outbreak investigation into the first identified human outbreak of cryptosporidiosis due to *Cryptosporidium cuniculus*, following a water quality incident in Northamptonshire, UK. Ethics permission was not required.

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Conflict of Interest Statements

Richard L. Puleston was previously a domestic water customer of Anglian Water.

Cathy M. Mallaghan is a domestic water customer of Anglian Water.

Deborah E. Modha has no conflicts of interest to declare.

Paul R. Hunter has no conflicts of interest to declare.

Jonathan S. Nguyen-Van-Tam is a domestic water customer of Anglian Water.

Christopher M. Regan has no conflicts of interest to declare.

Gordon L. Nichols has no conflicts of interest to declare.

Rachel M. Chalmers has no conflicts of interest to declare.

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